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HYALURONIC ACID AND THE RHEUMATIC DISEASES

The mechanism of the activity of rheumatic diseases is still unknown. There seems to be, however, an increasing amount of evidence linking the connective tissue in general and the interfibrillar material in particular with rheumatic diseases. Histologically and chemically, connective tissue consists of two distinct components, the fibrillar material and the interfibrillar substances. In the latter two components can be distinguished: the amorphous and viscous ground substance and the cement substance proper. The chemistry of the ground substance and cement substance is still not completely known. Two compounds, hyaluronic acid and chondroitin-sulfuric acid, have been isolated which probably occur singly or together in varying proportions in all connective tissue. The biology and chemistry of hyaluronic acid, as far as they are known today, have been reviewed recently. Chondroitin sulfates, though known much longer than hyaluronic acid, have been studied much less extensively. They are compounds of high molecular weights, composed of acetylgalactosamine sulfate and uronic acids, which have been isolated from hyalin cartilage, aorta, umbilical cord and skin. Recently a sulfate ester, which also appears to be chondroitin sulfate, has been isolated from tendon, although rigid proof of this has not been obtained thus far. The chondroitin sulfates as obtained from different sources, show differences in optical rotation, in sensitivity to alkali and in the rate of hydrolysis by hyaluronidase or an enzyme associated with hyaluronidase in testicular extracts. Whether these differences are real or due to differences in their preparation cannot be decided with certainty at present. Although the chondroitin sulfate of hyaline cartilage can be extracted with neutral or slightly alkaline salt solutions, especially calcium chloride, extraction of chondroitin sulfate from connective tissues requires strong alkali. Furthermore the polysaccharide of hyaline cartilage is easily dissociated from the accompanying protein, while extracts obtained from skin, tendon and umbilical cord yield the free mucopolysaccharide acids only after treatment with strong alkali. Without such treatment, the polysaccharides are obtained as protein complexes, in which the protein seems to be firmly bound to the polysaccharides. It appears from histochemical studies (Wislocki and Dempsey and associates) that chondroitin sulfate occurs in the intima of arteries. This layer may even continue to the precapillaries and capillaries. That this zone does exist in the capillary wall is suggested by the increase in capillary permeability caused by hyaluronidase injected into the connective tissue, whereas intravenous injection had no effect (Chambers). The enzyme (furnished by this laboratory) in vitro attacked chondroitin sulfate.

The concept of the rheumatic diseases as primarily diseases of the cement substances goes back to the histologic studies of Klinge, although this author did not distinguish clearly between collagenous fibers and cement substances. In the following brief statements an attempt will be made to point out the evidence which may possibly link the cement substances to the rheumatic diseases. To be sure, this evidence is not conclusive and especially fails to account for the differences in the various rheumatic diseases. The data on cement substances in relation to rheumatic diseases appears suggestive, however, and warrants a continued study.

The highest concentrations of hyaluronic acid in the mammalian body are found in synovial fluid and the skin and next in vitreous humor. It is still not known with certainty which cells produce the acid, but it appears that young fibroblasts in undifferentiated connective tissue may produce it in large quantities. One tissue which is now being studied in this connection is the young callus formed in healing experimental fractures.

The only micro-organisms known to produce hyaluronic acid are groups A and C hemolytic streptococci in the mucoid phase. Nonmucoid hemolytic streptococci may produce hyaluronidase as do many other micro-organisms, such as pneumococci, staphylococci and some gas-producing anaerobes. It appears significant that following an outbreak of sore throat caused by type 4 streptococci in a convalescent home for rheumatic children, Kuttner observed no recrudescence of rheumatic activity, while in previous years, sore throat due to other types of hemolytic streptococci led to a definite number of recurrences. According to Crowley, types 4 and 22 were the only strains of streptococci which

ANNUAL MEETING

The Annual Meeting and Twenty-first Scientific Sessions of the American Heart Association will be held in Chicago, Illinois, on June 18th and 19th, 1948. The Hotel Stevens will be the headquarters for all meetings. The first Scientific Session will be held on Friday afternoon, June 18th.

Business sessions will be scheduled Friday morning and on Sunday. The Annual Dinner is scheduled for Saturday evening, June 19th, at the Stevens.

Hotel rooms will be in great demand and every member who wishes to attend is urged to make reservations immediately by writing directly to the Reservations Manager of the Stevens.

produced hyaluronidase in demonstrable quantities. The strain of group A hemolytic streptococci, which in this institution was first used to demonstrate the presence of hyaluronidase, belonged to type 4. All attempts to grow this strain of streptococci in a mucoid form failed. Group C hemolytic streptococci also produce hyaluronic acid and many strains produce hyaluronidase. Apparently no data exist which link the group C streptococci to rheumatic fever. The hyaluronidase-producing streptococci all show a peculiar variability in the activity of the enzyme, a variability not found in other hyaluronidase-producing cocci. The activity disappears, either because of inhibition or enzymatic destruction. This inhibition deserves further study.

Hyaluronic acid has been isolated from normal synovial fluids of animals and abnormal synovial fluids from man. Synovial fluid from the knees of normal human beings could not be obtained in sufficient quantity for chemical isolation. The normal fluids, on dilution, acidification and addition of normal horse serum, form a fibrous clot, whereas under the same conditions, most pathologic fluids go into a state of colloidal turbidity. On addition of 0.01 unit of hyaluronidase, an amount insufficient to affect the viscosity or concentration of hyaluronic acid of the material under the experimental conditions, the fiber formation is prevented and the colloidal turbidity seen in pathologic fluids occurs.

A quantitative difference between normal and pathologic fluids also was found. This difference could best be expressed by a factor derived from the log of the viscosity of the fluid divided by the concentration of hyaluronic acid in the fluid determined turbidimetrically. In fluids from normal joints this factor was always more than 10, while in fluids from patients who had rheumatoid arthritis or rheumatic fever, the factor was as low as 4. The activity of the disease was roughly inversely proportional to this factor; the more active the disease, the lower the factor and as the activity diminished, the factor rose.

Some information about the rheumatic process may be obtained from the effects of antirheumatic drugs. A possible relationship between the salicylates and the hyaluronic acid system was suggested by Guerra, who reported on the inhibition by salicylate of the spreading reaction of testicular hyaluronidase in animals and in man. This observation has been confirmed by this and other laboratories. This inhibition of the spreading reaction could not be due to the action of the salicylate on hyaluronidase, since salicylate in vitro inhibited hyaluronidase only in concentrations in which many biologically active proteins are denatured. The cause of the interference with the spreading reaction could be due to interference with the substrates or interference of a metabolic product of salicylate with the enzyme. Interference with the substrate may be suggested from the strong influence of salicylate-feeding on excretion of glucuronic acid. The biosynthesis of hyaluronic acid and chondroitin sulfate from glucuronic acid and acetylhexosamines is unknown. The inhibitory action of a biological derivative of salicylate on hyaluronidase has been definitely established. In our laboratory, strong inhibition of the action of hyaluronidase was found with gentisuric acid isolated from the urine of patients receiving salicylates. Synthetic gentisic acid also inhibited hyaluronidase to about 50 per cent in 0.0005 molar concentration. The inhibitory effect of gentisic acid is apparent only when the acid and the enzyme are incubated together, while the copresence of substrate prevents this inactivation. Meanwhile, Loewenstein and Gagnon reported in a short note that the quinone of gentisic acid inhibited hyaluronidase. In this laboratory other water-soluble quinones and hydroquinones were found to be strong inhibitors of hyaluronidase, among them homogentisic quinone, 1,4-naphthoquinone-2-sulfonic acid and polyporic acid, while benzohydroquinone and quinone and 1,2-naphthoquinone-4-sulfonic acid were inactive. Cysteine, ascorbic acid, acetyl-glucosamine and glucuronic acid could not replace hyaluronic acid in the prevention of the inactivation.

That the antirheumatic effects of salicylates is solely due to the inhibition of hyaluronidase does not seem likely, and other enzyme systems may be involved. However, the hyaluronic acid in the synovial fluid is influenced by the oral administration of salicylates. The factor described previously rises after the administration of salicylates. Salicylates and other antirheumatic drugs (amidopyrine, which also inhibits the spreading reaction) may act by influencing the synthesis of normally occurring quinones, especially benzoquinone acetic acid (the quinone of homogentisic acid).

The nonspecific inhibition of hyaluronidase by human serums is now under investigation by a number of laboratories. It has been reported that the serums of rheumatic patients as well as of patients who have sore throats due to hemolytic streptococci have an inhibitory power greater than the serums of normal persons. This inhibition appears to be a complex and difficult problem, depending on relative concentrations of hyaluronidase and serum and on the presence of inorganic ions, such as chloride and phosphate. At present there seems to be no clear-cut indication that this inactivation of hyaluronidase by human serums has any significance in rheumatic disease.

COMMENT

In the preceding brief outline the emphasis has been placed on hyaluronic acid, while changes in the chondroitin sulfates and their enzymatic breakdown have hardly been mentioned. It may well be that changes in the chondroitin sulfates and in their protein complexes are of greater importance for the rheumatic processes than those associated with hyaluronic acid. It seems possible that the feature distinguishing one rheumatic disease from another is the mucopolysaccharide-enzyme system which is primarily involved in a particular disease. The study of the interstitial mucopolysaccharides and their relation not only to the rheumatic diseases but also to the problem of growth and differentiation of aging and malignancy promises to be a rewarding field of investigation.

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